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# Elevated Carbon Dioxide Level Suppresses Nutritional Quality of Lettuce and Spinach

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## Abstract

**Rising global $CO_2$ levels are a major factor that impacts not only the environment but also many plant functions including growth, productivity and nutritional quality. The study examined the impact of elevated [$CO_2$] on nutritional quality and growth characteristics of lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*). Elevated [$CO_2$] decreased the concentration of many important nutrients including nitrogen (protein), potassium and phosphorus in the edible parts of both lettuce and spinach. The nitrogen concentration in lettuce shoots was reduced by more than 30% at elevated [$CO_2$] compared to the plants grown at ambient level of $CO_2$. Similarly the concentration of a number of micronutrients including sulfur, zinc, copper and magnesium, was depressed in lettuce shoots. Although the total phenolic content and antioxidant capacity were higher in lettuce at elevated $CO_2$, they were not affected in spinach. The photosynthetic activity was variable among the plant species while there was no increase in the carbon accumulation in these plants at elevated [$CO_2$]. However, there was significant reduction in the leaf stomatal conductance in both lettuce and spinach in response to higher [$CO_2$], which is likely affect both water loss from the leaves and their photosynthetic activity. The results indicate a broad adverse impact of rising [$CO_2$] on the nutritional quality of commonly consumed leafy vegetables namely, lettuce and spinach.**

## Keywords

**Elevated Carbon Dioxide, Lettuce, Nutritional Quality, Phytochemicals, Protein Deficiency, Spinach**

## 1. Introduction

A major part of our changing environment is the rapid and steady increase in the global atmospheric $CO_2$ concentration ([$CO_2$]) which is predicted to continue to rise to dangerous levels in the coming decades [1]. The ris-

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ing [$CO_2$] levels affect not only the climate but also directly the crop growth and productivity and thus, the global food security. A number of studies have examined the impact of elevated [$CO_2$] on plant growth and production [2] [3]. The consensus appears to be that elevated [$CO_2$] is likely to increase the biomass and yield [4]-[7], which is related to an increase photosynthetic rate in plants [8]. However, this increase in photosynthetic activity is short lived and not sustained over a long term exposure to elevated $CO_2$. Such plants tend to acclimate to high levels of $CO_2$ and become less responsive [9]. Another important effect of elevated $CO_2$ is its ability to decrease plants' stomatal conductance thereby reducing their transpiration and improving their water use efficiency leading to improved drought resistance [10] [11]. Thus, these studies indicate that there has been a greater focus on the effect of rising $CO_2$ levels on plant growth, function and productivity; however, very little attention has been given to its possible impact on the nutritional quality of crops. Furthermore, by and large, studies relating nutritional quality remain mostly centered around field crops. Relatively, there are only a few studies dealing with the nutritional quality of fruits and vegetables as affected by elevated [$CO_2$] despite their importance in human health. In recent decades, fruits and vegetables are being actively promoted as a significant part of a healthy diet because they are typically rich in nutrients and health-promoting phytochemicals, and thus their daily consumption can not only reduce the risk of chronic and degenerative diseases but also provide a sense of overall well-being [12]-[15].

While the rising $CO_2$ levels are likely to increase the yield of many crops [16] [17], they can also lower their protein content [18]. There is a growing consensus on the negative impact of higher [$CO_2$] on the nitrogen accumulation in field crops, however, its impact on the overall nutritional quality reflecting other important nutrients and phytochemicals still remains inconclusive [19]. Recently, in an extensive meta-analysis to examine the effect of elevated [$CO_2$] on nutrient content of several varieties of 6 commonly cultivated field crops, Myers *et al*. [18] showed that major $C_3$ cereal crops including rice and wheat had lower concentration of iron and zinc, in addition to protein, compared to their counter parts grown at ambient $CO_2$ level. Thus, the reduced concentration of these important nutrients in major cereal crops (rice and wheat) and legumes (field peas and soybean) which are the staple crops in many developing countries certainly raises the specter of a wide spread global malnutrition.

Elevated [$CO_2$] can also affect the health-promoting phytochemicals in plants which have not been adequately explored. There have been some reports that show that elevated $CO_2$ may increase the photosynthetic activity in plants favoring higher C-N ratio which is likely to have a positive impact on the synthesis of secondary metabolites many of which are known to function as health-promoting phytochemicals and plant defense compounds [20]-[23]. In this study, we examine the effect of elevated [$CO_2$] on the nutritionally important nutrients, health-promoting phytochemicals and the growth and development of popular leafy vegetables, namely lettuce and spinach.

## 2. Materials and Methods

### 2.1. Plant Growing Conditions

*Lactuca sativa* (Lettuce, var. Black-Seeded Simpson) and *Spinacia oleracea* (Spinach, var. Bloomsdale Long Standing) were used as model plants in this study. Seeds (W. Atlee Burpee & Co., PA) were germinated in flats (11" W × 21.37" L × 2.44" D) containing Premier Promix soil medium (Hummert International, KS) in a controlled environmental chamber maintained at 280 - 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (photosynthetically active radiation), 12-hour photoperiod, and 18/20˚C (night/day) temperature with 400 ppm of $CO_2$ (control). After initiation of true leaves, plants were fertilized with 20-10-20 nutrient solution Peters Peat-Lite (Hummert International, KS) at 200 ppm constant liquid feed rate. Plants were watered every other day and fed with fertilizer solution once a week. Each seedling, at 2 - 4 leaf stage, was transplanted to individual pot (4" diameter) containing the same soil mix as indicated above. Plants were grown for 5 days before applying the $CO_2$ treatments. They were transferred to a Conviron $CO_2$ growth chamber (CMP 6050 Control System) set at the same growing conditions as described above but at 700 ppm. Plants were randomly assigned to the treatments on a completely randomized design with 12 replications and were moved within each chamber once in two days to minimize the variability of environmental conditions within the growth chambers. Growth chamber temperature was checked periodically to make sure that it was maintained at the desired level. The concentration of $CO_2$ was monitored using an infrared gas analyzer (LiCOR Model 6400, NE).

### 2.2. Macro and Micro Nutrients

The concentration of each macro- and micro-mineral nutrients was determined using ground dry shoot and root samples. Concentration of phosphorus, potassium, calcium, magnesium, zinc, iron, copper, manganese and sulfur ($SO_4$) was determined by perchloric digest method as outlined by Gieseking *et al*. [24]. The nutrient concentration was quantified from the perchloric digest using an Inductively Coupled Plasma (ICP) Spectrometer (Model 720-ES ICP Optical Emission Spectrometer, Varian Australia Pty Ltd., Australia). Total carbon and nitrogen content (organic and inorganic) in samples was determined using LECO TruSpec carbon/nitrogen combustion analysis (LECO Corp., MI).

### 2.3. Total Phenolics

Total phenolic compounds were extracted according to Oh *et al*. [20] using a modified Folin Ciocalteu reagent method. One gram of leaf tissue each from 6 replicates (in each treatment) was ground in 6 mL of 80% acetone and then 1.5 mL of the sample was transferred to a centrifuge tube and kept in the darkness overnight at 4˚C. The extract was than centrifuged at 1000 rpm for 5 min and a 50 µL of the supernatant was mixed with 135 µL of water, 750 µL diluted (1:10) Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) and 600 µL of 7.5% (w/v) $Na_2CO_3$. The mixture was vortexed and incubated in water bath at 45˚C for 15 min and was then allowed to cool at room temperature. Absorbance was read at 765 nm (U-1100 spectrophotometer, Hitachi Ltd. Japan). Gallic acid standards were prepared from freshly prepared gallic acid 1mg/mL gallic acid (Acros Organics, Belgium) in 80% acetone with 3 replicates for each concentration.

### 2.4. Total Antioxidants

Total Antioxidants were measured using ABTS decolorization assay as outlined by Miller and Rice-Evans [25] and Pennycooke *et al*. [26]. A 5 mM ABTS stock solution was prepared in 20 mL distilled water and the ABTS* radical cations were generated by adding 1 g of $MnO_2$ as an oxidizing agent to ABTS solution and stirring continuously at room temperature. Excess $MnO_2$ was removed by filtering through a Buchner funnel under vacuum first, and then using 0.2 µM syringe end filter. Then the ABTS* solution was diluted to an absorbance value of 0.7 (±0.02) at 734 nm by using 5 mM PBS (phosphate buffer saline) at pH 7.4 and stored in a water bath at 30˚C. Trolox standards were prepared from a stock solution of 0.5 mM trolox. One mL of ABTS* reagent was added to trolox standards or samples and vortexed for 10 s and followed by 1 min of reaction time. The absorbance of the reaction mixture was measured at 734 nm. A PBS solution was used as a blank for each assay. The antioxidant capacity of samples was estimated as the mean value of trolox equivalent.

### 2.5. Plant Growth Measurements

Growth performance of plants was monitored by measuring plant height and leaf number after 10 and 17 days of transplanting in lettuce and every week in spinach. Leaf area measurement was conducted immediately after harvesting. The plants were separated into leaves, stem and roots. Out of 12 total replicates, 6 replicate samples were immediately frozen for biochemical analysis and another set of 6 samples was harvested for biomass and to measure leaf area. To measure leaf area, leaves were scanned (4 replicates for lettuce and 6 replicates for spinach) using a scanner (Epson Perfection V700 Scanner, Seiko Epson Corporation, Japan) and analyzed by using an image analysis system, winFOLIA (Regents instruments Inc. Canada). The total biomass of plants was determined by measuring fresh weight followed by dry weight, obtained by drying the samples in an oven for 72 hours at 70˚C. Similarly, shoot and root samples were dried and ground for nutrient content analyses.

### 2.6. Photosynthetic Efficiency and Stomatal Conductance

Steady state net photosynthesis ($P_n$) and stomatal conductance ($g_s$) were measured using an infrared gas analyzer (LiCOR, Model 6400, NE) equipped with a 6 $cm^2$ leaf cuvette with controlled environmental conditions ($CO_2$, light and temperature). Measurements were made after 2 weeks of treatment in lettuce and after 3 weeks in both lettuce and spinach following the manufacturer's guidelines. Recently fully expanded leaves receiving direct light were used for the study.

### 2.7. Leaf Chlorophyll

Newly matured leaves (0.35 g) were ground in 3 mL of cold acetone in a chilled mortar and pestle in the dark. Samples were centrifuged at 4000 rpm for 10 min and the supernatant was pipetted into another tube. The pellet was washed 3 times with 3 mL of cold acetone followed by centrifugation. The extract was combined to make a final volume of 12 mL and its absorbance was read at 663 nm, 646 nm, 710 nm and 652 nm and *Chl a*, *b* and total chlorophyll were determined using the following relationships [27]:

*Chl a* (mg/mL) = 12.21 ($A_{663}$-$A_{710}$) - 2.81($A_{646}$-$A_{710}$)

*Chl b* (mg/mL) = 20.13 ($A_{646}$-$A_{710}$) - 5.03($A_{663}$-$A_{710}$)

Total *Chl* (mg/mL) = 27.8 ($A_{652}$-$A_{710}$)

### 2.8. Statistical Analyses

The experiments were conducted using a completely randomized design with 12 replications and the treatments were separated using student's t-test with *p*-value threshold set at 0.05. The treatment means with standard error are presented with significant differences indicated at 0.01 or 0.05 level.

## 3. Results and Discussion

Growing lettuce and spinach at elevated [$CO_2$] reduced the accumulation of many nutrients important for human nutrition both in their shoots and roots. In edible parts of lettuce (shoots), nitrogen, phosphorus, potassium, sulfur, magnesium, copper and zinc levels were significantly lower at high [$CO_2$] compared to ambient [$CO_2$] (**Figure 1** and **Figure 2**). The percent changes in the concentrations for the nutrients in shoots and roots due to elevated [$CO_2$] in relation to control are presented in **Figure 2** and **Figure 4** for lettuce and spinach respectively. The concentration

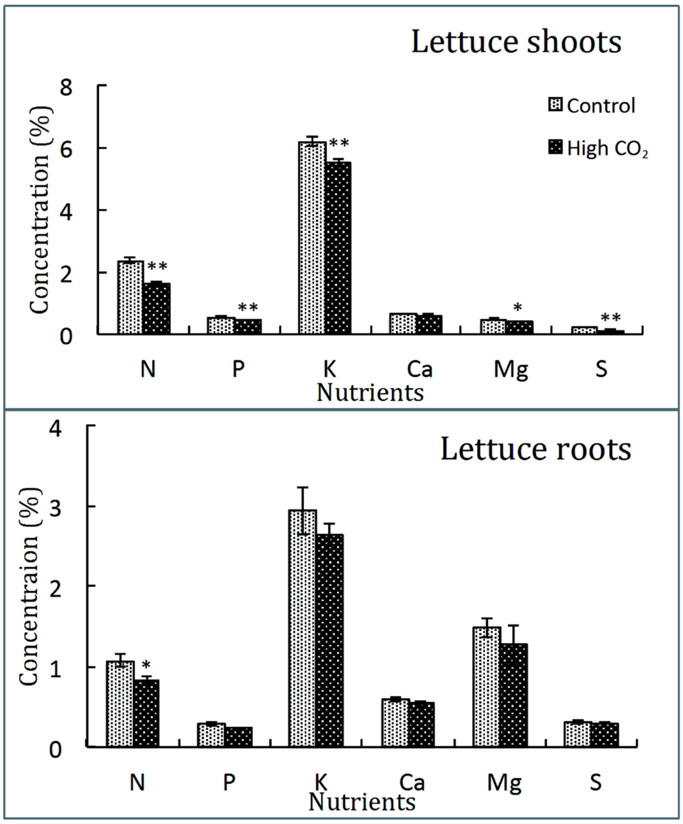


**Figure 1.** Nutrient concentration in lettuce at plants ambient and elevated [$CO_2$]. Lettuce plants were grown at ambient (control) and elevated level of $CO_2$ (700 ppm). Levels of major nutrients in shoots and roots were measured after harvest. Means with standard error bars and significant differences at $p < 0.05$ ($^*$) and $p < 0.01$ ($^{**}$) are presented.

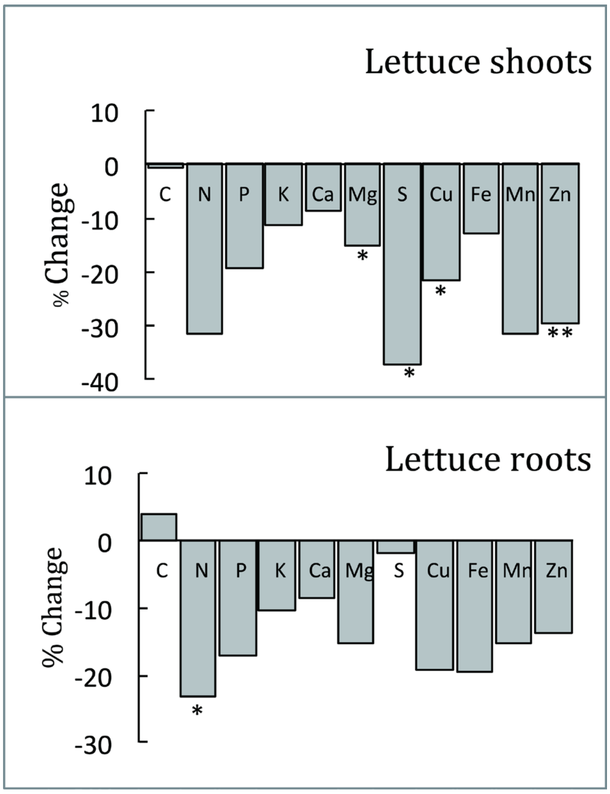


**Figure 2.** Percent change in nutrient concentrations in lettuce in response to elevated [$CO_2$]. The changes in the nutrient concentration relative to the controls in shoots and roots of lettuce caused by elevated [$CO_2$] are presented. Significant differences are indicated at $p < 0.05$ ($^*$) and $p < 0.01$ ($^{**}$).

of nitrogen and sulfur in lettuce shoots was reduced by more than 30% and that of copper and zinc by more than 20% relative to the plants grown at ambient [$CO_2$] (**Figure 2**). In spinach shoots, there was also a general decline in the level of macro and micro nutrients important in human diet at elevated [$CO_2$]. Significant decrease in nitrogen, phosphorus and potassium concentration occurred in the shoots grown at elevated [$CO_2$] (**Figure 3**). Nitrogen concentration in both shoots and roots of lettuce was depressed while it was only lower in spinach shoots but not in roots. The only major nutrient that accumulated at higher concentration at elevated [$CO_2$] was calcium in spinach shoots, which was about 17% higher than in those grown at the ambient $CO_2$ level (**Figure 4**). However, carbon accumulation in shoots and roots of both lettuce and spinach was not affected by elevated [$CO_2$].

There is overwhelming evidence that elevated [$CO_2$] can suppress not only protein content but also various other important nutrients in many wide ranging field crops [18]. Reduced protein content in food crops has serious implication especially in developing countries in Africa, Asia and Latin America not only among general population but also among young children where protein deficiency can lead to stunted growth and increased vulnerability to many diseases [28] [29]. In a number of $C_3$ cereal and legume crops, Myers *et al.* [18] found that elevated [$CO_2$] under field conditions significantly depressed the level of protein, iron and zinc in the edible portions of these crops. The reason for low protein concentration in these crops could be due to interference of nitrogen (especially nitrate) assimilation by high [$CO_2$] [30]. In addition, the general decrease in nutrient content in plants grown at elevated [$CO_2$] is often thought to be due to dilution caused by high carbon accumulation [31]. This may not be the case because the decline in individual nutrient concentrations at elevated [$CO_2$] is quite variable [18]. For example, in this study elevated [$CO_2$] actually increased the calcium concentration in spinach shoots while concentration of other nutrients either decreased or was not affected. Furthermore, it should be noted that in this study elevated [$CO_2$] did not have any significant effect on the carbon accumulation in both lettuce and spinach (**Figure 2** and **Figure 4**).

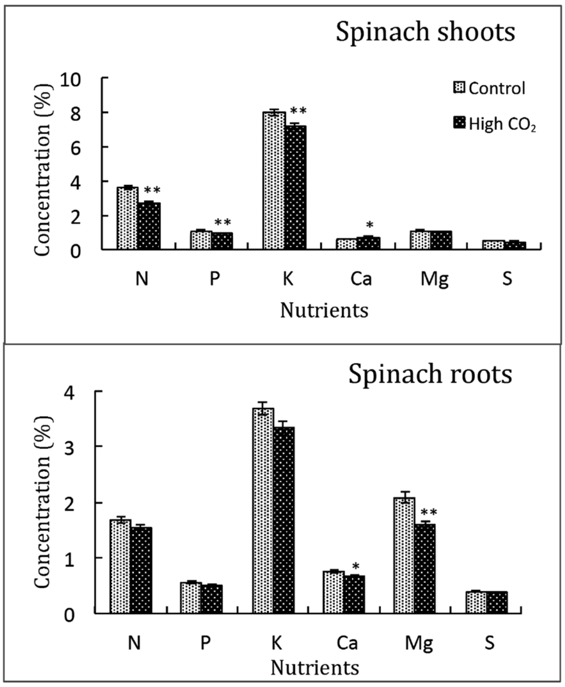


**Figure 3.** Nutrient concentration in spinach at ambient and elevated [$CO_2$]. Spinach plants were grown at ambient and elevated level of $CO_2$. Levels of major nutrients in shoots and roots were measured in the shoots and roots after harvest. Means with standard error bars and significant differences at $p < 0.05$ ($^*$) and $p < 0.01$ ($^{**}$) are presented.

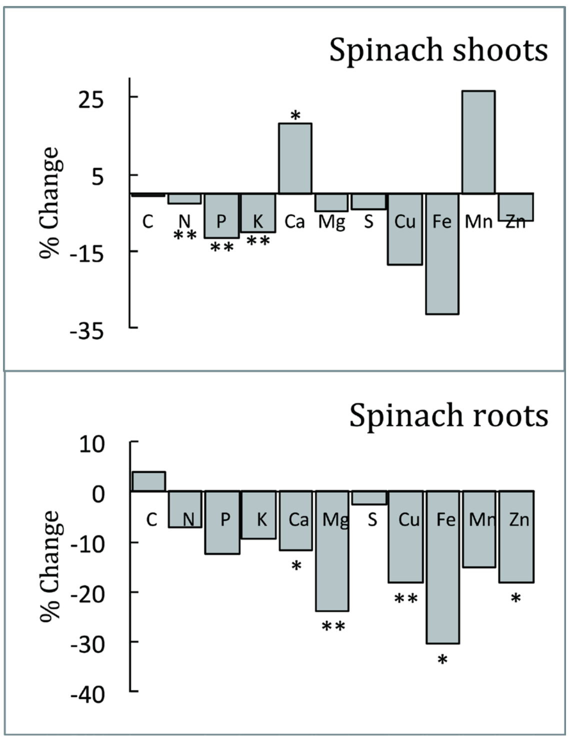


**Figure 4.** Percent change in nutrient concentrations in spinach in response to elevated [$CO_2$]. The changes in the nutrient concentration relative to the controls in shoots and roots of spinach caused by elevated [$CO_2$] are presented. Significant differences are indicated at $p < 0.05$ ($^*$) and $p < 0.01$ ($^{**}$).

At elevated [$CO_2$], the decline in the accumulation of protein, zinc and iron in many $C_3$ crops can cause serious problems to a large portion of global population which depends on these crops to meet the dietary requirements of these nutrients [18]. We found that zinc concentration was suppressed by nearly 30% in lettuce shoots and potassium level reduced significantly both in lettuce and spinach shoots by elevated [$CO_2$]. Zinc deficiency is globally a common problem especially in sub-Saharan Africa and South Asia, about 17.3% of global population is at risk of being afflicted by this problem [30]. Potassium is another nutrient that is likely to be deficient in human diet and is an important electrolyte in human body and plays a vital role in muscle function, hydration and in regulating blood pressure [32]. The concentration of sulfur, a vital nutrient involved in many cellular oxidation-reduction reactions and in the formation collagen, a protein found in connective tissues, was also reduced by more than 37% in lettuce shoots. Considering that most of the global population depends on a few restricted number of staple crops to meet its nutritional requirement, poor nutrient content in these crops is likely to be a major concern as it can lead to a wide spread global health problems [18] [33].

A similar trend of depressed nutrient concentrations with regard to both macro and micro nutrients was observed in roots of these crops as well. At elevated [$CO_2$], nitrogen concentration was significantly decreased in lettuce roots, while levels of a number of nutrients including calcium, magnesium, copper, iron and zinc were lower in spinach roots.

It is interesting to note that the concentration of all the major plant nutrients (nitrogen, phosphorus and potassium) was much higher in the shoots than in the roots of both crops at ambient and elevated $CO_2$ levels, indicating they are highly mobile within the plants, and shoots tend to accumulate these nutrients preferentially. Potassium is known to be highly mobile within the plants, and thus, both lettuce and spinach shoots had the highest concentration of this among all other mineral nutrients. This is consistent with the observation that plants tend to take up potassium in excess of what is actually needed for their growth, known as luxury consumption [34]. Also, spinach shoots had a higher concentration of zinc, three times of that observed in the roots, although zinc is not known to be a highly mobile nutrient (**Table 1**). In contrast, as iron is relatively immobile nutrient, very little of what was taken up by the roots was translocated to the shoots, as indicated by its very high concentration in roots both in lettuce and spinach and only less than 2.5% of what was taken up by the roots was present in the shoots.

In lettuce leaves, the total phenolic content and the total antioxidant capacity were much higher at elevated [$CO_2$] (**Figure 5**). Compared to plants grown at ambient [$CO_2$], the total phenolic content and the total antioxidant capacity in the leaves increased by about 63% and 49% respectively at elevated [$CO_2$]. However, in contrast, there was no significant difference in either the total phenolic content or the total antioxidant capacity

**Table 1.** The mineral nutrient concentrations of shoots and roots of lettuce and spinach grown at ambient and elevated [$CO_2$]. Means with standard errors and significant differences at $p < 0.05$ ($^*$) and $p < 0.01$ ($^{**}$) are presented.

| | Concentration (ppm) | | | |
|---|---|---|---|---|
| | Shoot | | Root | |
| Lettuce | Control | High [$CO_2$] | Control | High [$CO_2$] |
| Cu | 6.06 ± 0.32 | 4.75 ± 40$^*$ | 16.73 ± 1.12 | 13.49 ± 1.83 |
| Fe | 108.01 ± 8.43 | 94.30 ± 5.18 | 6696.95 ± 546.96 | 5380.26 ± 1326.43 |
| Mn | 168.70 2 ± 1.46 | 115.59 ± 8.51 | 176.97 ± 16.40 | 149.77 ± 21.24 |
| Zn | 42.19 ± 2.03 | 29.78 ± 0.89$^{**}$ | 32.37 ± 1.075 | 27.92 ± 3.30 |
| Spinach | | | | |
| Cu | 12.721 ± 0.85 | 10.38 ± 0.90 | 24.08 ± 0.81 | 19.68 ± 0.31$^{**}$ |
| Fe | 161.07 ± 33.39 | 110.63 ± 5.80 | 7273. 68 ± 598.60 | 5057.65 ± 442.67$^*$ |
| Mn | 146.14 ± 12.57 | 184.57 ± 19.80 | 201.65 ± 14.93 | 171.46 ± 9.15 |
| Zn | 219.88 ± 11.56 | 203.76 ± 90 | 68.55 ± 4.25 | 56.00 ± 3.37$^*$ |

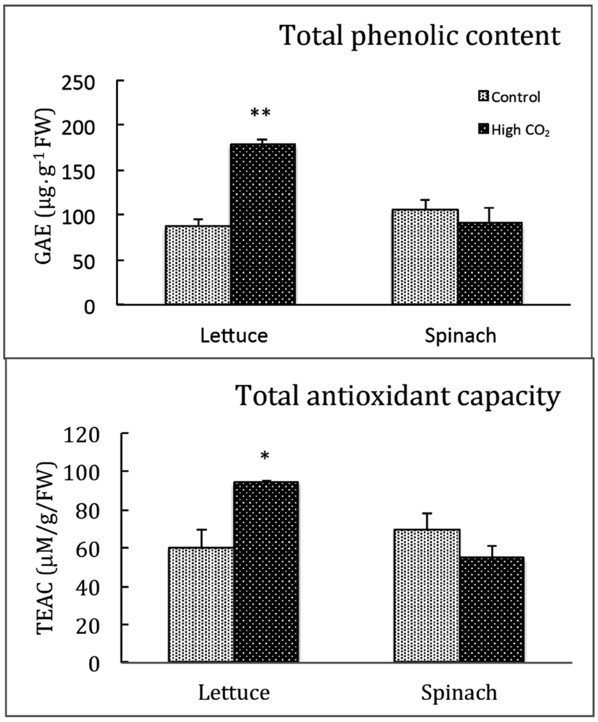


**Figure 5.** Total phenolic content and total antioxidant capacity in lettuce and spinach at ambient and elevated [$CO_2$]. The total phenolic content (as gallic acid equivalent) and total antioxidant capacity (as trolox equivalent) were determined after harvest in lettuce and spinach shoots from plants grown at ambient and elevated [$CO_2$]. Means with standard error bars and significant differences at $p < 0.05$ ($^*$) and $p < 0.01$ ($^{**}$) are presented.

between spinach plants grown at ambient and elevated $CO_2$ levels (**Figure 5**). The higher total phenolics in lettuce was also noted by Perez-Lopez *et al*. [35] in two lettuce cultivars along with total glutathione and carotenoids content at elevated CO2 level. Similarly, Zhang *et al*. [23] showed that elevated [$CO_2$] could improve health-promoting and sensory qualities of tomato fruits grown in greenhouse. High [$CO_2$] favors increased photosynthetic activity in many plant species favoring higher C-N ratio which can have a positive impact on the carbon based phytochemicals such as phenolic compounds and their derivatives in plants. Wu *et al*. [21] found that elevated [$CO_2$] increased the C-N ratio in leaves of cotton plants along with tannins and gossypol, a phenolic aldehyde commonly found in cotton plants.

In addition to the nutritional quality of these crops, we also examined their plant growth characteristics at ambient and elevated $CO_2$ conditions. At elevated [$CO_2$], there was significant increase in the dry biomass accumulation in lettuce, more than 18% over the ambient $CO_2$ level (**Figure 6**). Similar results have been observed in lettuce especially with shoot biomass [36] but the results are variable depending on the cultivar [35]. Although lettuce plants grown at elevated [$CO_2$] increased their biomass, their overall leaf area decreased with much greener and thicker leaves compared to their counterparts grown at ambient [$CO_2$]. Both shoot and root biomass in lettuce increased at high [$CO_2$], however, the root growth appeared to be relatively higher than the shoot growth at elevated [$CO_2$] resulting in lower shoot-root biomass ratio (data not shown). At elevated [$CO_2$], although there was no difference in biomass, there was a significant decrease in leaf area in spinach between these two growing conditions, just as in lettuce plants. Also, no significant difference in plant height and leaf number either in lettuce or spinach was observed (data not presented).

The net photosynthetic rate of young lettuce plants (at 2-week stage) was much higher, more than 2-fold at elevated [$CO_2$], than at ambient [$CO_2$] (**Figure 7**). However, with longer exposure (for 3 weeks) to high [$CO_2$], the net photosynthetic rate declined well below the control plants (at ambient [$CO_2$]). Similar observations have been made in a number of plant species wherein long term exposures to higher [$CO_2$] resulted in attenuation of the typical positive response of growth and photosynthesis as the plants acclimate to the elevated levels [6] [8]

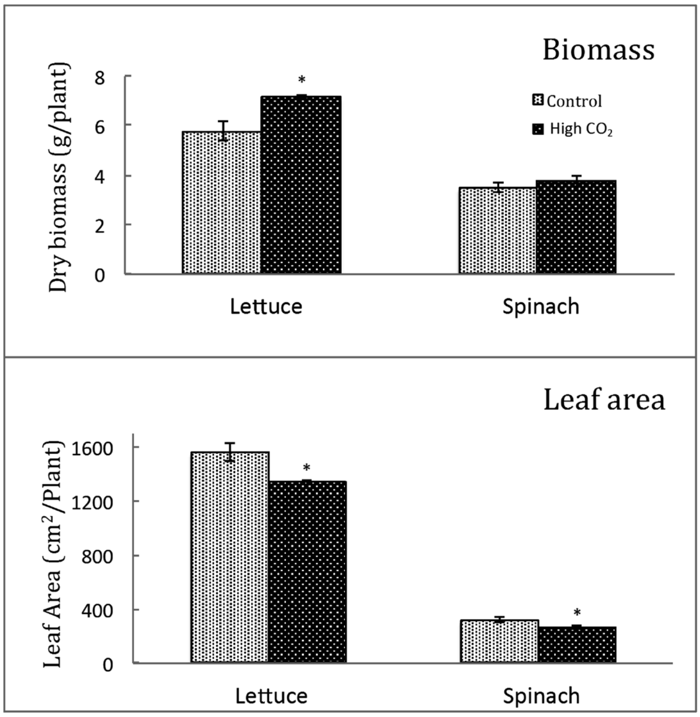


**Figure 6.** Biomass accumulation and leaf area in lettuce and spinach at ambient and elevated [$CO_2$]. Biomass (dry weight basis) and leaf area of individual plants of lettuce and spinach were determined after harvest. Means with standard error bars and significant differences at $p < 0.05$ ($^*$) are presented.

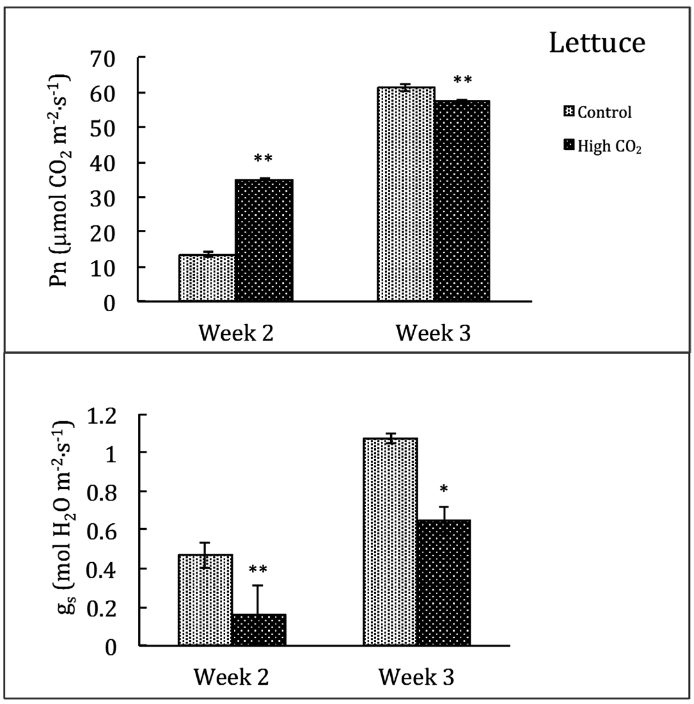


**Figure 7.** Photosynthetic activity and stomatal conductance in lettuce at ambient and elevated [$CO_2$]. The net photosynthesis (*Pn*) and stomatal conductance ($g_s$) were measured on fully expanded leaves after 2 and 3 weeks of treating plants with elevated [$CO_2$]. Means with standard error bars and significant differences at $p < 0.05$ ($^*$) and $p < 0.01$ ($^{**}$) are presented.

[30] [37]-[39]. Similarly, a study aimed at examining the partition of carbon in oak seedlings at elevated [$CO_2$] showed that the dry matter accumulation was higher in the beginning of the season but drastically decreased as reflected by the decrease in the photosynthetic activity toward the end of the season [40]. The decline in photosynthetic activity over the long term appears to be due to a decrease in nitrogen content and the amount of $CO_2$ fixing enzyme, Rubisco, in the leaves. In actively growing leaves, Rubisco is a major nitrogen containing compound in the leaves and is often the rate limiting enzyme in carbon assimilation. As elevated $CO_2$ levels suppress nitrogen assimilation in plants, long term exposure exposure of plants to elevated [$CO_2$] results in reducing the Rubisco content and its efficiency in fixing $CO_2$ [8] [41]. Similar to older lettuce plants, photosynthetic efficiency of spinach plants was not affected by elevated [CO2] (**Figure 8**). In addition, leaf total chlorophyll content both in lettuce and spinach was not affected by higher [$CO_2$] (**Table 2**). Although some studies have shown a decrease in chlorophyll content in lettuce at elevated [$CO_2$] [36] the results seem quite variable [35].

Elevated [$CO_2$] reduced the stomatal conductance by nearly 60% and 65% in lettuce and spinach, respectively (at 3 week stage) compared to ambient [$CO_2$] (**Figure 7** and **Figure 8**). This is consistent with previous studies which show that elevated [$CO_2$] increases water use efficiency of plants by reducing stomatal conductance and transpiration rates in several plant species [6] [36] [38] [42]. This is likely to have an impact not only on water

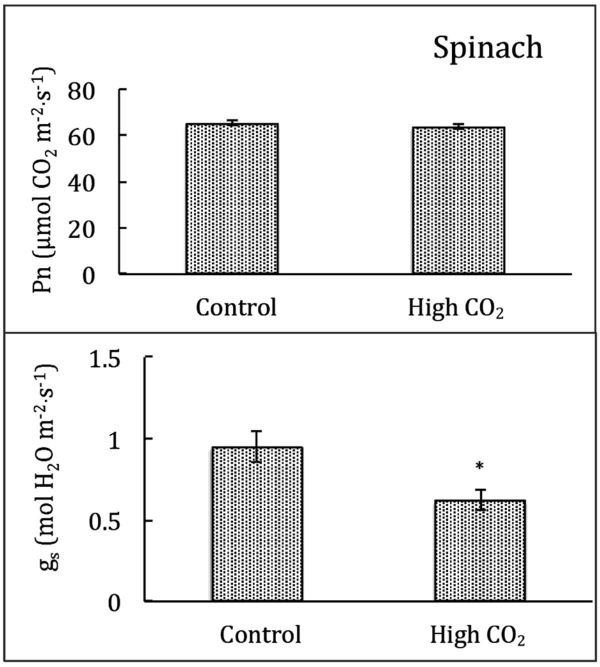


**Figure 8.** Photosynthetic activity and stomatal conductance in spinach at ambient and elevated [$CO_2$]. The net photosynthesis (Pn) and stomatal conductance ($g_s$) were measured on fully expanded leaves after 3 weeks of treating plants with elevated [$CO_2$]. Means with standard error bars and significant differences at $p < 0.05$ ($^*$) are presented.

**Table 2.** Leaf chlorophyll content of lettuce and spinach plants grown at ambient and elevated [$CO_2$] determined at the time of harvest and expressed as mg/g FW. Means with standard errors are presented.

| | *Chl a* | *Chl b* | Total *Chl* |
|---|---|---|---|
| Lettuce | | | |
| Control | 7.17 ± 2.04 | 17.61 ± 1.29 | 109 ± 4.14 |
| High [$CO_2$] | 6.56 ± 0.47 | 27.77 ± 6.32 | 136 ± 14.00 |
| Spinach | | | |
| Control | 4.76 ± 0.54 | 17.57 ± 0.68 | 82.89 ± 3.26 |
| High [$CO_2$] | 4.11 ± 0.61 | 17.74 ± 2.48 | 80.07 ± 11.42 |

status but also on the photosynthetic activity of these plants. Thus, one of the contributing factor for the decreased photosynthetic rate observed in lettuce plants at higher [$CO_2$] may be their reduced stomatal conductance and leaf area, which could affect not only water loss from the plants but also the influx of $CO_2$ into the leaves.

## 4. Conclusion

Rising atmospheric $CO_2$ levels can have a negative impact on the nutritional quality of lettuce and spinach, two commonly consumed leafy vegetables. Elevated [$CO_2$] depresses the concentration of a number of nutrients which are important for human nutrition in the edible parts of these plants. It decreases the concentration of nitrogen (protein), phosphorus and potassium along with many important micronutrients including copper, zinc, magnesium and sulfur in either lettuce, spinach or both. The significant decline in nutritional quality in leafy vegetables at high [$CO_2$] may portend a potential for a wide-spread dietary nutrient deficiency especially as fruits and vegetables are becoming a major part of our daily diet. However in response to high [$CO_2$], the total phenolic content and antioxidant capacity which are linked to health-promoting qualities increase in lettuce but not in spinach. At elevated [$CO_2$]. Thus, there is a decrease in stomatal conductance and leaf area with no effect on carbon accumulation. The results indicate that rising $CO_2$ levels can compromise the general nutritional quality by depressing the concentration of many essential nutrients in commonly consumed leafy vegetables such as lettuce and spinach.

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